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(54) Title: MEASLES IMMUNIZATION BY DNA TRANSCRIPTION UNIT INOCULATION

(57) Abstract

This invention relates to methods and compositions for immunizing a mammal against measles virus, comprising introducing into the mammal at least one DNA transcription unit comprising DNA encoding a measles virus antigen operatively linked to DNA which is a promoter region, resulting in the expression of the antigen, thereby eliciting an immune response. The elicited immune response can provide protection against disease caused by the measles virus. The host can be any mammal, including a human.

cytotoxic T lymphocyte assays. The cytotoxic T cells have specificity not only for measles virus surface antigens, such as H and F, but also for internal antigens, especially the N protein.

No effective inactivated vaccine is yet available, but live attenuated vaccines are widely used. Attenuation of the wild-type human measles virus, the Edmonston strain, is achieved by its adaptation to and serial passage in various cell lines, leading to a mutant whose activity is partially restricted in humans. One problem with the use of this vaccine is that immunized infants often retain maternally derived antibodies which restrict replication of the live measles virus, decreasing its immunogenicity, and, therefore, decreasing the efficacy of the vaccine. This is particularly problematic in developing countries where measles infection and mortality rates are high in infants under one year of age, an age where they are likely to retain maternal measles neutralizing antibodies. The live attenuated vaccines are also limited in that they do not raise as high, or as long-lived, neutralizing antibody responses as wild-type infections. It would be advantageous to have additional vaccines against measles available.

Summary of the Invention

This invention relates to methods and compositions for immunization against the measles virus using subunit vaccination. Specifically, this invention relates to a method of immunizing a mammal against the measles virus, comprising introducing into the mammal a DNA transcription unit (or units). DNA transcription units are taken up and expressed in the host cells of the mammal, resulting in production of the measles antigen or antigens. An immune response, such as a humoral immune response, a cell-mediated immune response or both is/are elicited in the mammal,

providing protection against measles infection. The host can be any mammal, including a human. The invention also relates to compositions comprising at least one DNA transcription unit and a pharmaceutically acceptable (physiologically acceptable) carrier. The invention also relates to DNA transcription units for use in the claimed methods and compositions.

Each DNA transcription unit used in the claimed methods and compositions comprises DNA encoding at least one measles virus antigen operatively linked to DNA which is a transcriptional promoter element or elements (the promoter region). The promoter region can be of retroviral or nonretroviral origin. Measles virus antigen(s) encoded by the DNA in the transcription unit are one or more of the following: hemagglutinin (H), matrix (M), fusion protein (F), nucleocapsid protein (N), large polymerase (L), phosphoprotein (P), and nonstructural protein (C) of the measles virus. Each DNA transcription unit can comprise multiple copies of the same antigen, and/or it can comprise different antigens. The antigens can be from the same or different strains of measles virus.

A DNA transcription unit can be used to express any measles virus antigen, such as hemagglutinin or fusion protein, as well as one or more antigenic fragments and/or peptides that have been experimentally determined to be effective in immunizing a mammal against infection by measles virus. Furthermore, a DNA transcription unit can be designed to produce internal, surface, secreted, or assembled forms of the antigens being used as immunogens. For example, the antigen can be in secreted form or a precursor form. Each antigen can be selected from a subset of T cell-recognized determinants or epitopes in a measles virus protein. Alternatively, or in addition, each antigen can be selected from a subset of B cell-recognized determinants or epitopes in a measles virus protein.

In one embodiment of the present invention, the antigen is a measles virus hemagglutinin protein. In another embodiment, the antigen is a measles virus fusion protein. The antigen can be a measles virus hemagglutinin protein in a secreted form, for example, sHA4, or in a transmembrane form, such as HA7.

In the claimed methods, a single DNA transcription unit or multiple DNA transcription units can be administered to a mammal to achieve immunization against one antigen or multiple antigens. Likewise, a composition can contain a single DNA transcription unit or multiple DNA transcription units, in addition to a physiologically acceptable carrier. Furthermore, the compositions and DNA transcription units can comprise different measles antigens, for example, measles antigens of different strains, and/or they can comprise antigens from pathogens or infectious agents other than those of the measles virus. In a preferred method embodiment, the mammal is inoculated with both F DNA and H DNA. In a preferred composition embodiment, the composition comprises both H DNA and F DNA.

The DNA transcription units and compositions can be administered through various routes, including the parental route or the mucosal route. The mucosal route can be oral or respiratory (including nasal and tracheal mucosal surfaces). Alternatively, the DNA transcription units and compositions can be administered through a route of administration selected from the group consisting of: intravenous, intramuscular, intraperitoneal, intradermal, and subcutaneous routes. The DNA transcription units can be administered in a pharmaceutically acceptable carrier. The DNA transcription unit or composition can be microsphere-encapsulated. In one embodiment, the DNA transcription unit or composition is coated onto gold beads for administration by particle bombardment delivery, for example, the gene gun.

In one embodiment of the present invention, the individual is immunized through one or more parenteral routes of inoculation. DNA transcription units administered to the skin can be delivered with a gene gun. In a second embodiment, the individual is immunized by contacting a mucosal surface, such as a respiratory mucosal surface or tracheal mucosal surface, with DNA transcription units in such a manner that the transcription units are taken up by (i.e., enter the cells of) the mucosal surface. DNA transcription units for mucosal administration can be microsphere-encapsulated.

There are numerous advantages to the current invention. For example, immunization can be accomplished for any antigen encoded by measles DNA. Furthermore, the encoded measles antigens are expressed as "pure" antigens which can undergo host cell modifications in a manner similar to the modifications undergone by antigens expressed by the wild type strain. In addition, the DNA is easily and inexpensively manipulated and is stable as a dry product or in solution over a wide range of temperatures. This is particularly useful in less-developed countries where refrigeration is unavailable. Moreover, the efficacy of subunit DNA vaccines is not reduced by the presence of persistent maternal antibodies in infants. Thus, this technology is valuable for the development of highly effective subunit vaccines against the measles virus.

Brief Description of the Drawings

Figure 1 is a schematic representation of the pJW4303 vector comprising SV40 origin (SV40 Ori), bovine growth hormone polyadenylation sequences (BGHpA), the Edmonston Hemagglutinin H and sH sequence inserts, and a CMV immediate promoter sequence (CMV Pro) which includes the sequence encoding the CMV

CLAIMS

1. A product for use in therapy in a mammal, e.g., immunization, and comprising a DNA transcription unit comprising DNA encoding an antigen of measles virus operatively linked to DNA which is a promoter region.

2. A product comprising a DNA transcription unit, wherein the transcription unit(s) comprise(s) DNA encoding an antigen of measles virus operatively linked to DNA which is a promoter region, for use in therapy, e.g. for use in immunization of a mammal.

3. Use of a DNA transcription unit comprising DNA encoding an antigen of measles virus operatively linked to DNA which is a promoter region, for the immunization of a mammal.

4. A method of immunizing a mammal, said method comprising administering to the mammal a DNA transcription unit comprising DNA encoding an antigen of measles virus operatively linked to DNA which is a promoter region.

5. A product comprising more than one DNA transcription unit, each transcription unit comprising DNA encoding an antigen of measles virus operatively linked to DNA which is a promoter region, wherein the antigen of measles virus for one transcription unit is different from the antigen of measles virus of the other transcription unit, or each of the other transcription units, for use in therapy, e.g. for use in immunization of a mammal.

6. A product for use in therapy comprising more than one DNA transcription unit, each transcription unit comprising DNA encoding an antigen of measles virus operatively linked to DNA which is a promoter region, wherein the antigen of measles virus for one transcription unit is different from the antigen of measles virus of the other transcription unit, or each of the other transcription units, e.g. for use in a method of immunizing a mammal.

7. Use of a product comprising more than one DNA transcription unit for immunizing a mammal by administering to said mammal the product, wherein each transcription unit comprises DNA encoding an antigen of measles virus operatively linked to DNA which is a promoter region, wherein the antigen of measles virus for one transcription unit is different from the antigen of measles virus of the other transcription unit, or each of the other transcription units.

8. A method of immunizing a mammal, said method comprising administering to a mammal more than one DNA transcription unit, each transcription unit comprising DNA encoding an antigen of measles virus operatively linked to DNA which is a promoter region, wherein the antigen for one transcription unit is different from the antigen of the other transcription unit, or each of the other transcription units.

9. A product, use or method according to any one of the preceding claims, wherein the promoter region, or each of the promoter regions, is/are of nonretroviral origin.

10. A product, use, or method according to any one of the preceding claims, wherein the promoter region, or each of the promoter regions, is/are of retroviral origin.

11. A product, use, or method according to any one of the preceding claims, wherein the antigen, or at least one antigen, is a measles virus hemagglutinin.

12. The product, use or method according to any one of the preceding claims, wherein the antigen, or at least one antigen, is a measles virus fusion protein.

13. The product, use or method of claim 12, wherein the measles virus fusion protein is F_o.

14. A product, use or method according to any one of the preceding claims wherein the antigen, or each of the antigens, is capable of eliciting a protective response against a disease caused by the measles virus.

15. A product, use or method according to claims 5-8 or 14 wherein each antigen is from a different strain of the measles virus.

16. A product, use or method according to claims 5-8 or 14 wherein each antigen is from a different measles virus protein.

17. A product, use or method according to any one of the preceding claims wherein the antigen, or each of the antigens, is a measles virus protein selected from the group consisting of: hemagglutinin, fusion protein, matrix protein,

nucleocapsid protein, phosphoprotein, large polymerase protein, and nonstructural protein.

18. A product, use or method according to claims 5-8 or 14, wherein each antigen is a measles virus hemagglutinin from a different strain of the measles virus.

19. A product, use or method according to claims 5-8 or 14 wherein each antigen is a measles virus fusion protein from a different strain of the measles virus.

20. A product, use or method according to claims 5-8 or 14 wherein at least one of the antigens is a measles virus hemagglutinin and at least one of the antigens is a measles virus fusion protein.

21. A product, use or method according to any one of the preceding claims, wherein the antigen or each of the antigens is a secreted form of a protein selected from the group consisting of: hemagglutinin and fusion protein.

22. A product, use or method according to any one of the preceding claims wherein the antigen, or each of the antigens, is selected from subset of T cell-recognized determinants in a measles virus protein.

23. A product, use or method according to any one of the preceding claims wherein the antigen, or each of the antigens, is selected from subset of the B-cell recognized epitopes in a measles virus protein.

24. The product, use or method according to any one of the preceding claims, wherein the transcription unit, or each of the transcription units, is microsphere encapsulated.

25. The product, use or method according to any one of the preceding claims, wherein the transcription unit, or each of the transcription units, is coated onto gold beads for administration to the mammal by particle bombardment delivery.

26. The product, use or method according to any one of the preceding claims wherein the transcription unit, or each of the transcription units, in a physiologically acceptable carrier, is administered to the mammal through a route of administration selected from the group consisting of intravenous, intramuscular, intraperitoneal, intradermal and subcutaneous.

27. The product, use or method according to any one of the preceding claims wherein the transcription unit, or each of the transcription units, in a physiologically acceptable carrier, is administered to the mammal parenterally.

28. The product, use or method according to any one of claims 1 to 25 wherein the transcription unit, or each of the transcription units, is administered to the mammal by contact to a mucosal surface.

29. The product, use or method according to claim 28, wherein the mucosal surface is a respiratory mucosal surface, such as a nasal mucosal surface or a tracheal mucosal surface.

30. The product, use or method of any of the preceding claims, wherein at least one transcription unit further comprises at least one antigen which is not from the measles virus.

31. The product, use or method of Claim 30, wherein at least one antigen is from a pathogen selected from the group consisting of: influenza, rotavirus tetanus, respiratory syncytial virus, diphtheria, pertussis, mumps and rubella.

32. The product, use or method of any one of the preceding claims, wherein the transcription unit is directly expressed by host cell factors.

33. The product, use or method of any one of the preceding claims, wherein the mammal is a human.

34. Use of a DNA transcription unit comprising DNA encoding an antigen of measles virus operatively linked to DNA which is a promoter region, for the manufacture of a medicament for use in immunization of a mammal.

35. Use of a product comprising more than one DNA transcription unit for the manufacture of a medicament for immunizing a mammal by administering to said mammal the product, wherein each transcription unit comprises DNA encoding an antigen of measles virus operatively linked to DNA which is a promoter region, wherein the antigen of measles virus for one transcription unit is different from the antigen of measles virus of the other transcription unit, or each of the other transcription units.

36. A product, use or method of any one of the preceding claims whereby a humoral immune response and/or cell-mediated immune response is/are elicited against the antigen or each of the antigens.

37. A method of immunizing a mammal against measles virus, said method comprising administering to the mammal a DNA transcription unit comprising DNA encoding a measles virus antigen of the virus operatively linked to DNA which is a promoter region, wherein the DNA transcription unit is expressed in cells of the mammal, whereby the mammal is protected from the measles disease.

38. The method of Claim 37, wherein the measles virus antigen is a measles virus hemagglutinin.

39. The method of Claim 38, wherein the measles virus hemagglutinin is HA7.

40. The method of Claim 38, wherein the measles virus hemagglutinin is sHA4.

41. The method of Claim 37, wherein the measles virus antigen is a measles virus fusion protein.

42. The method of Claim 41, wherein the measles virus fusion protein is F_o.

43. A method of immunizing a mammal against measles virus, said method comprising administering to the mammal one or more DNA transcription units, each comprising DNA encoding an antigen or antigens of the measles virus operatively linked to DNA which is a promoter region, wherein the DNA transcription unit or units are expressed in cells

of the mammal, thereby eliciting a humoral immune response, a cell-mediated immune response or both, against the antigen or antigens, whereby the mammal is protected from the measles disease.

44. The method of Claim 33, wherein the DNA transcription unit is administered in combination with one or more additional DNA transcription units, each comprising DNA encoding a different antigen of the measles virus operatively linked to a promoter region.

45. A composition comprising at least one DNA transcription unit comprising DNA encoding an antigen of measles virus operatively linked to DNA which is a promoter region, and a physiologically acceptable carrier.